

## New Species and Eight Undescribed Species Belonging to the Families Aspergillaceae and Trichocomaceae in Korea

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### ABSTRACT

During a survey of fungal diversity associated with insects, mud, soil, and freshwater niches in different areas in Korea, nine interesting fungal strains were isolated. Based on their morphological characteristics and molecular phylogeny analyses, using a combined data set of  $\beta$ -tubulin (*BenA*), calmodulin (*CaM*), and second largest subunit of RNA polymerase (*RPB2*) sequences, the strains CNUFC AM-44, CNUFC JCW3-4, CNUFC S708, CNUFC WT202, CNUFC AS1-29, CNUFC JCW3-5, CNUFC JDP37, and CNUFC JDP62 were identified as *Aspergillus alabamensis*, *A. floridensis*, *A. subversicolor*, *Penicillium flavigenum*, *P. laevigatum*, *P. lenticrescens*, *Talaromyces adpressus*, and *T. beijingensis*, respectively. The strain CNUFC JT1301 belongs to series *Westlingiorum* in section *Citrina* and is phylogenetically related to *P. manginii*. However, slow growth when cultivated on CYA, MEA, CREA is observed and the property can be used to easily distinguish the new species from these species. Additionally, *P. manginii* is known to produce sclerotia, while CNUFC JT1301 strain does not. Herein, the new fungal species is proposed as *P. aquadulcis* sp. nov. Eight species, *A. alabamensis*, *A. floridensis*, *A. subversicolor*, *P. flavigenum*, *P. laevigatum*, *P. lenticrescens*, *T. adpressus*, and *T. beijingensis*, have not been previously reported in Korea. The present study expands the known distribution of fungal species belonging to the families Aspergillaceae and Trichocomaceae in Korea.

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## 1. Introduction

*Aspergillus*, *Penicillium*, and *Talaromyces* are well-known species of the Aspergillaceae and Trichocomaceae families, have a worldwide distribution, and have been isolated from a diverse range of habitats from air to soil, to vegetation and indoor environments [1–3]. Many species of *Aspergillus*, *Penicillium*, and *Talaromyces* are important both in industry and medicine, whereas others are associated with food spoilage, mycotoxin production, and dangerous human and plant diseases [4,5]. Various species can grow at low or high temperatures, low pH values, high salt or sugar concentrations, or low oxygen levels [6,7].

The genus *Aspergillus* was first introduced by Micheli in 1729 [8] to describe asexual fungi whose conidiophores resembled an aspergillum. Later, in 1768, Haller [9] validated the genus; in 1832, Fries [10] sanctioned the generic name, with *Aspergillus glaucus* (L.) Link [= *Mucor glaucus* L.  $\equiv$  *Monilia glauca* (L.) Pers.] as the generic type. Members of this genus produce many different compounds, which are used or have potential to be used in pharmacology and biotechnology [1,5]. *Aspergillus* is

subdivided into 6 subgenera, 27 sections, and 75 series [11]. Currently, this genus consists of 446 species [11].

In addition, in 1809, Link [12] established the genus *Penicillium*, with the generic type *P. expansum*, to accommodate asexual fungi that bore *Penicillium* (painter's brush)-like fruiting bodies. They are also known for their ability to produce antibiotics, organic acids, alcohols, enzymes, pharmaceuticals, and other metabolites including mycotoxins [2,4,6]. This genus is divided into 2 subgenera, 32 sections, and 89 series [11]. Currently, the genus contains 483 accepted species [11].

The genus *Talaromyces* was established by Benjamin (1955) [13] for a teleomorph of *Penicillium*, with *Talaromyces vermiculatus* (= *T. flavus*) as the type species. These species are characterized by cleistothecial or gymnothecial ascomata, unitunicate 8-spored asci, and unicellular ascospores with or without equatorial crests. The anamorphs have predominantly biverticillate or rarely terverticillate conidiophores with acerose phialides and narrow column [3,14]. The genus is divided into seven sections based on multigene phylogenetic, morphological, and physiological characteristics reported by

**Table 1.** Source information and GenBank accession numbers of strains used in this study.

Species	Strain number	Source	GenBank accession no.			
			ITS	<i>BenA</i>	<i>CaM</i>	<i>RPB2</i>
<i>A. alabamensis</i>	CNUFC AM-44 = IMYKFGC000000011	Mud	MZ687461	OK105085	OK105086	OK431605
<i>A. floridensis</i>	CNUFC JCW3-4 = IMYKFGC000000038	Freshwater	MZ687462	OK105087		OK431606
<i>A. subversicolor</i>	CNUFC S708 = QWJQFGC0000000311	Bee	MZ687463	OK105083		OK105084
<i>P. aquadulcis</i>	CNUFC JT1301 = NNIBRFG31625	Freshwater	OK356194	OK105100	OK105102	
<i>P. aquadulcis</i>	CNUFC JT1302	Freshwater	OK356195	OK105101	OK105103	
<i>P. flavigenum</i>	CNUFC WT202 = NNIBRFG31617	Freshwater	MZ687464	OK105094	OK105095	OK105096
<i>P. laevigatum</i>	CNUFC AS1-29 = IMYKFGC000000010	Soil	MZ687465	OK105097	OK105098	
<i>P. lenticrescens</i>	CNUFC JCW3-5 = IMYKFGC000000039	Freshwater	MZ687466	OK105099		OK539663
<i>T. adpressus</i>	CNUFC JDP37 = QWJQFGC0000000435	Freshwater	MZ687467	OK105091	OK105092	OK105093
<i>T. beijingensis</i>	CNUFC JDP62 = QWJQFGC0000000436	Freshwater	MZ687468	OK105088	OK105089	OK105090

Yilmaz *et al.* [3]. Recently, a new section *Tenues* was proposed by Sun *et al.* [15]. Currently, the genus contains 171 accepted species [11]. Although the number of new species in the genera *Aspergillus*, *Penicillium*, and *Talaromyces* has increased in Korea, this number is significantly lower than the global number of accepted species. In addition, some habitats such as freshwater and insect hosts are still poorly studied.

The objective of this study was to morphologically and molecularly characterize of a new species, *P. aquadulcis*, and eight previously unrecorded fungal species in Korea—namely *A. alabamensis*, *A. floridensis*, *A. subversicolor*, *P. flavigenum*, *P. laevigatum*, *P. lenticrescens*, *T. adpressus*, and *T. beijingensis*—isolated from freshwater, insects, mud, and soil.

## 2. Materials and methods

### 2.1. Sample collection and isolation

Freshwater, insect, mud and soil samples were collected from different areas in Korea. The samples were collected in sterile plastic bags or sterile 50 mL Falcon tubes and transferred to the laboratory. To isolate fungi from freshwater, soil, and mud, a serial dilution plating method was used. Briefly, 1 mL of water or 1 g of soil or mud was mixed with 9 mL of sterile distilled water; serial dilutions ranging from  $10^{-1}$  to  $10^{-5}$  were prepared. An aliquot of 0.1 mL from each dilution was transferred to potato dextrose agar (PDA; Difco, Becton, Dickinson and Co., Sparks, MD, USA) or yeast malt extract agar (YMA; Difco) supplemented with the antibiotic streptomycin sulfate (100 ppm) and was incubated at 25 °C for 3–7 days. To isolate pure cultures, individual colonies with various morphologies were picked, transferred to PDA, and subcultured until pure mycelia were obtained. Fungal isolation from insect samples were conducted following our previous methods [16]. All pure isolates were maintained in PDA slant tubes and in 20% glycerol at –80 °C at the Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, Korea as CNUFC AM-44, CNUFC JCW3-

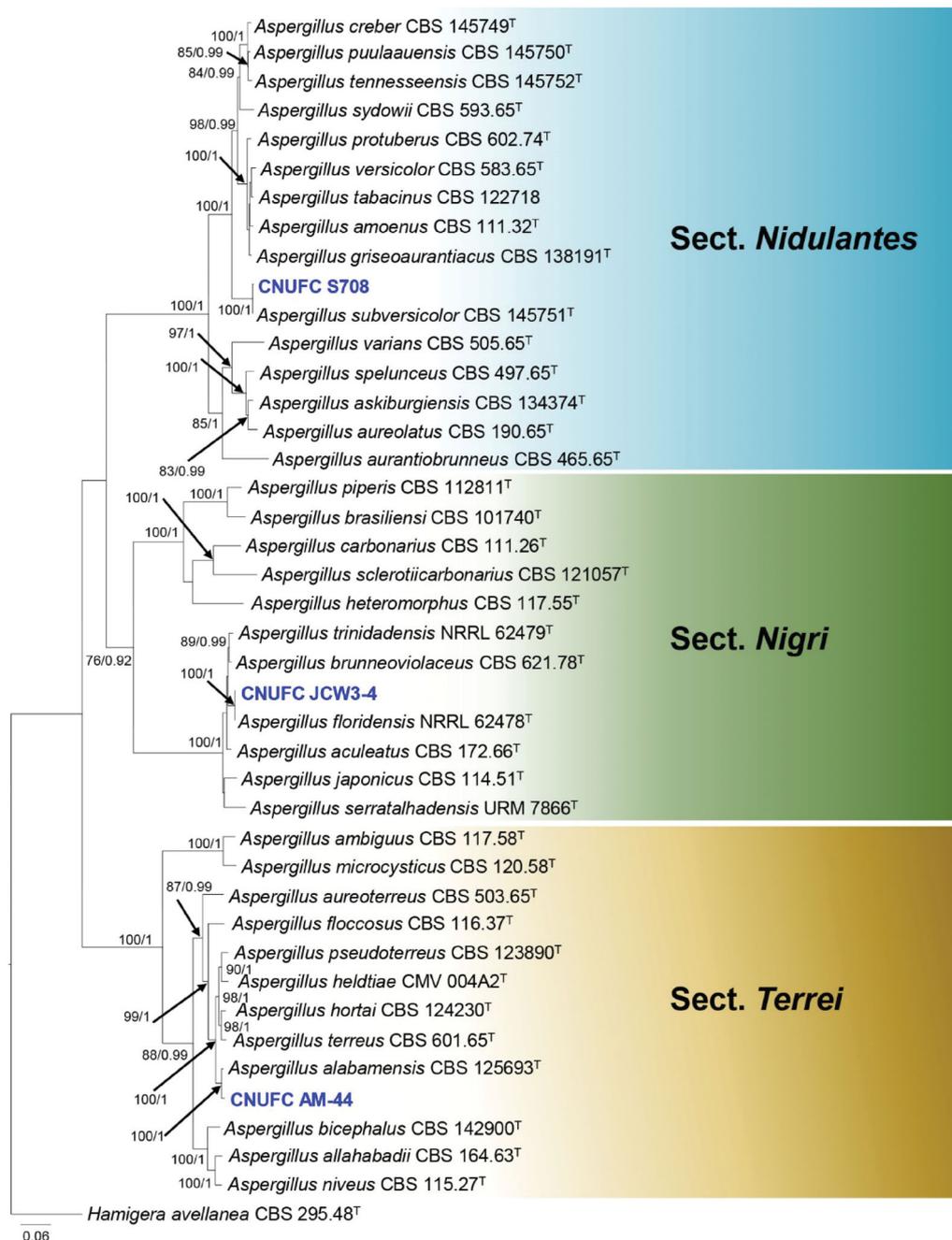
4, CNUFC S708, CNUFC JT1301, CNUFC WT202, CNUFC AS1-29, CNUFC JCW3-5, CNUFC JDP37, and CNUFC JDP62. The isolates were also deposited at the Collection of National Institute of Biological Resources (NIBR) Incheon, and Nakdonggang National Institute of Biological Resources (NNIBR), Sangju, Korea. Type specimens were deposited in Chonnam National University Herbarium, Gwangju, Korea as inactive dried cultures. Information on all isolates used in this study is shown in Table 1.

### 2.2. Morphological characteristics

The strains were three-point inoculated onto Czapek yeast autolysate agar (CYA; yeast extract, Difco), malt extract agar (MEA; Difco), yeast extract sucrose agar (YES; yeast extract, Difco), except creatinine sucrose agar (CREA) only for CNUFC JT1301 strain. All petri dishes were inoculated at 25 °C for 7 days. Medium preparation, inoculation, and incubation were performed according to the methods reported by Yilmaz *et al.* [3]. Colony characteristics were recorded after 7 days. Lactic acid (60%) was used as the mount fluid, and 96% ethanol was used to remove excess conidia. The cultures were examined and photographed under an Olympus BX53 light microscope with an attached Olympus DP74 camera (Olympus, Tokyo, Japan). For SEM, samples were performed as described previously by Nguyen *et al.* [17]. The samples were then sputter-coated with gold and imaged with scanning electron microscope (Hitachi S4700 Field-Emission SEM, Hitachi, Tokyo, Japan) at the Korea Basic Science Institute, Gwangju, Korea.

### 2.3. DNA extraction, PCR, and sequencing

Fungal isolates were cultured on PDA overlaid with cellophane at 25 °C for 5–7 days. Genomic DNA was extracted using the Solg TM Genomic DNA Preparation Kit (Solgent Co. Ltd., Daejeon, Korea). The internal transcribed spacer (ITS) region was amplified using the primer pairs V9G and ITS4 [18,19].  $\beta$ -tubulin (*BenA*) was amplified using the primer pairs T10 and Bt2b [20]. Calmodulin (*CaM*)

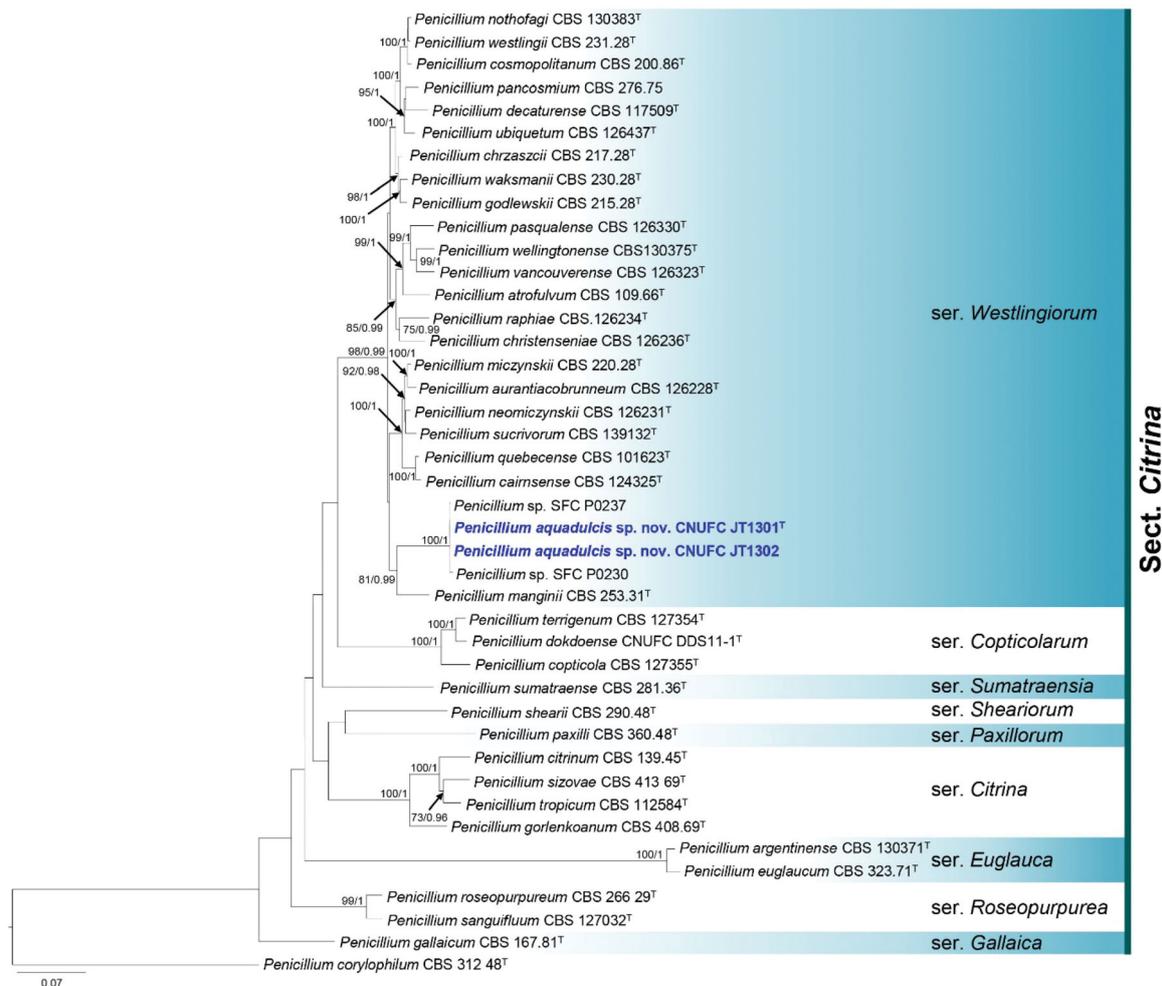


**Figure 1.** Phylogram generated from the Maximum Likelihood (RAxML) analysis based on a combined *BenA*, *CaM* and *RPB2* sequence data for species classified in the *Aspergillus* sections *Nigri*, *Terrei* and *Nidulantes*. Bootstrap values  $\geq 70\%$  MLBS and  $\geq 0.90$  BPP are indicated above or below branches. *Hamigera avellanea* CBS 295.48 was used as the outgroup. The newly generated sequences are indicated in blue bold. T = ex type.

was amplified using the primer pairs CF1 and CF4 [21,22]. Second largest subunit of RNA polymerase (*RPB2*) was amplified using the primer pairs RPB2-5F and RPB2-7cR [23]. PCR amplification was performed according to the conditions described by Visagie *et al.* [2] and Yilmaz *et al.* [3]. The PCR products were then purified using an Accuprep PCR Purification Kit (Bioneer Corp., Daejeon, Korea) and sequenced on the ABI PRISM 3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the same primers employed for PCR.

#### 2.4. Phylogenetic analysis

Sequences for the selected strains were retrieved from the GenBank and aligned using MAFFT (<http://mafft.cbrc.jp/alignment/server>) with the algorithm L-INS-I. Aligned sequences were automatically trimmed using trimAl [24] using the gappyout method. Concatenated datasets of the *BenA*, *CaM* and *RPB2* genetic markers were built using MEGA7 [25]. Phylogenetic reconstructions by maximum likelihood (ML) was carried out using RAxML-HPC2 on XSEDE on the online CIPRES Portal (<https://www.phylo.org/portal2>) with bootstrap



**Figure 2.** Phylogram generated from the Maximum Likelihood (RAxML) analysis based on a combined *BenA*, *CaM* and *RPB2* sequence data for species classified in *Penicillium* section *Citrina*. Bootstrap values  $\geq 70\%$  MLBS and  $\geq 0.90$  BPP are indicated above or below branches. *Penicillium corylophilum* CBS 312.48 was used as outgroup. The newly generated sequence is indicated in blue bold. T = ex type.

support obtained from 1,000 replicates. The GTR + GAMMA model of nucleotide evolution was used. Bayesian inference analysis was performed with MrBayes 3.2.2 [26], using a Markov Chain Monte Carlo (MCMC) algorithm. The sample frequency was set to 100 and the first 25% of trees were removed as burn-in. Support values are provided at the branches [ML bootstrap support (MLBS), BI posterior probability (BPP)]. *Hamigera avellanea* CBS 295.48 was chosen as the outgroup in the *Aspergillus* and *Penicillium* phylogenies. *Penicillium corylophilum* CBS 312.48 was chosen as the outgroup in the section *Citrina*. *Talaromyces varians* CBS 386.48 was the outgroup for the phylogeny of *Talaromyces*. The newly obtained sequences have been deposited in the GenBank database under the accession numbers provided in Table 1.

### 3. Results

#### 3.1. Molecular phylogeny

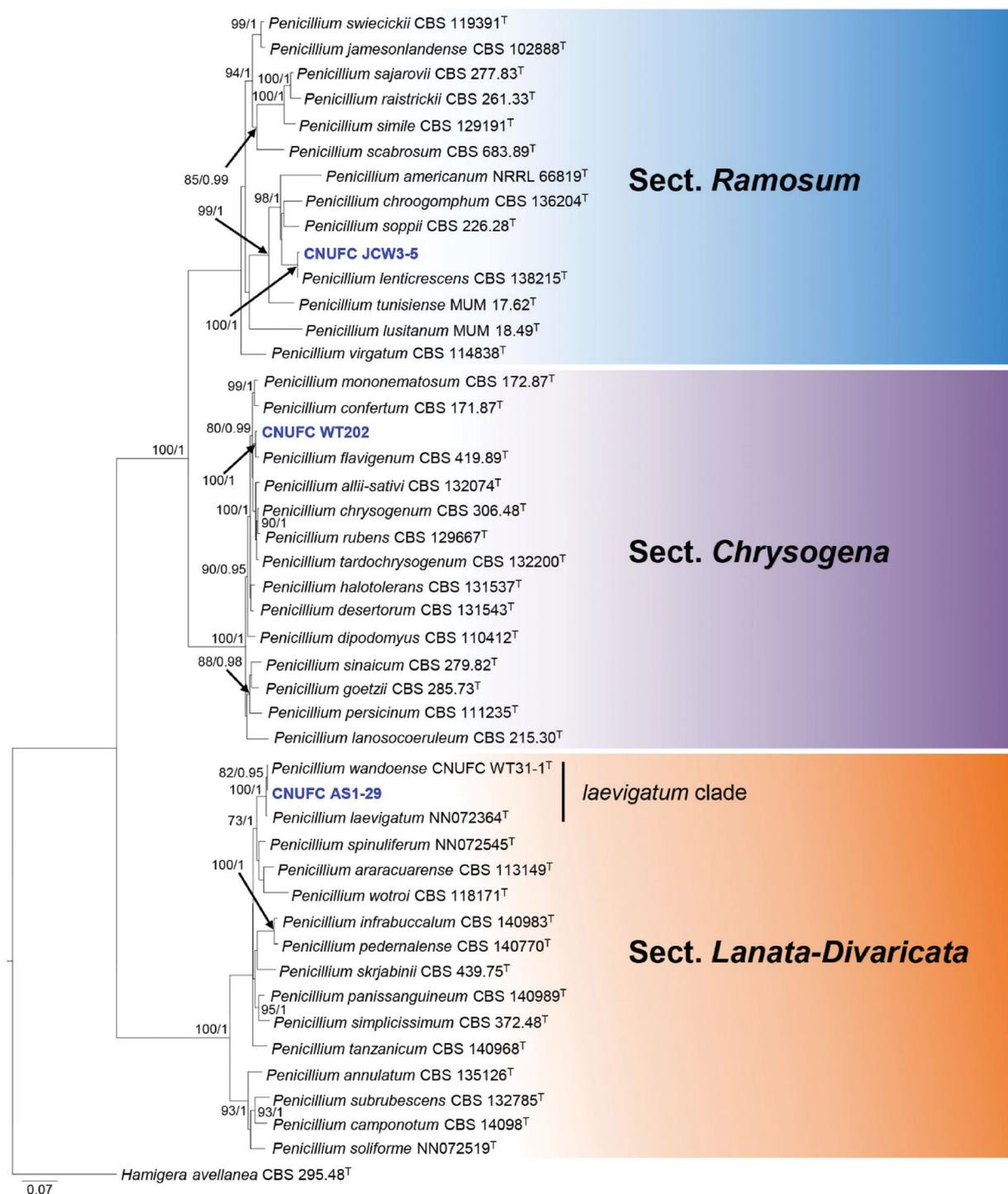
Combined *BenA*, *CaM* and *RPB2* was used to clarify the phylogenetic relationships of newly collected

specimens with other species in the genera *Aspergillus*, *Penicillium*, and *Talaromyces* (Figures 1–4).

The three-locus phylogenetic tree with the 41 isolates of *Aspergillus* is shown in Figure 1. The concatenated alignment consists of 2,227 characters (including alignment gaps): 541, 676, and 1,010 characters used in the *BenA*, *CaM*, and *RPB2*, respectively. The isolates CNUFC S708, CNUFC JCW3-4, and CNUFC AM-44 are clustered within the same clade as *Aspergillus subversicolor* CBS 145751 (ex-type strain), *A. floridensis* NRRL 62478 (ex-type strain), and *A. alabamensis* CBS 125693 (ex-type strain), in the sections *Nidulantes*, *Nigri*, and *Terrei*, respectively.

The phylogenetic trees generated by the concatenated *BenA*-*CaM*-*RPB2* sequences (424, 528, 915 and 1,867 characters with gaps, respectively) indicates that our two isolates CNUFC JT1301 and CNUFC JT1302 are related to *P. manginii* with high statistical support (Figure 2).

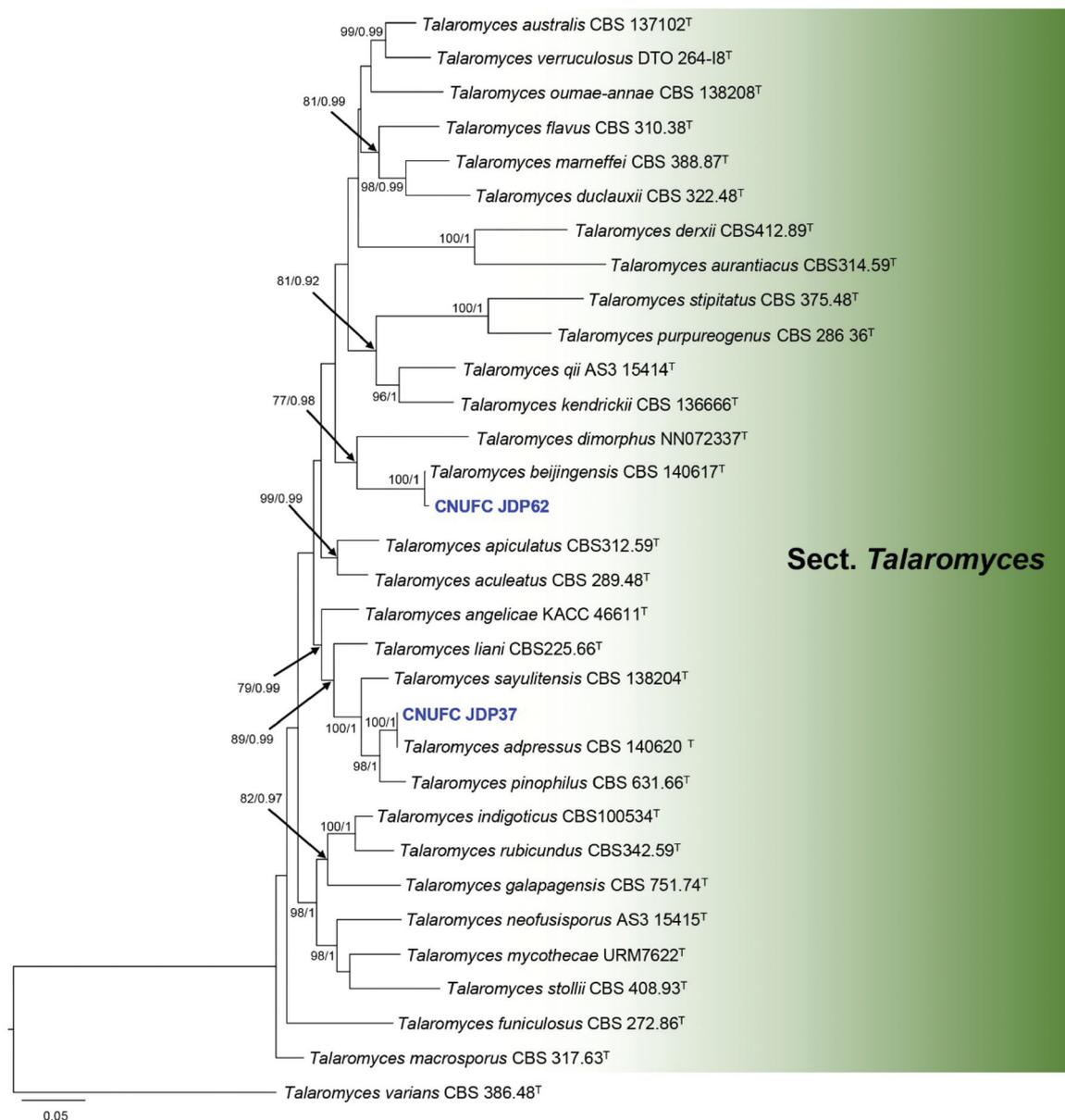
The phylogenetic tree based on the three-locus with the 45 isolates of *Penicillium* is shown in Figure 3. The concatenated alignment consists of



**Figure 3.** Phylogram generated from the Maximum Likelihood (RAxML) analysis based on a combined *BenA*, *CaM* and *RPB2* sequence data for species classified in the *Penicillium* sections *Chrysogena*, *Lanata-Divaricata* and *Ramosum*. Bootstrap values  $\geq 70\%$  MLBS and  $\geq 0.90$  BPP are indicated above or below branches. *Hamigera avellanea* CBS 295.48 was used as the out-group. The newly generated sequences are indicated in blue bold. T = ex type.

1,918 characters (including alignment gaps): 422, 551, and 945 characters used in the *BenA*, *CaM*, and *RPB2*, respectively. The isolates CNUFC WT202, CNUFC AS1-29, CNUFC JCW3-5 are clustered within the same clade as *P. flavigenum* CBS 419.89, *P. laevigatum* NN072364, and *P. lenticrescens* CBS 138215, in the sections *Chrysogena*, *Lanata-Divaricata*, and *Ramosum*, respectively.

The phylogenetic tree based on the three-locus (Figure 4) shows the relationships of the two *Talaromyces* isolates included in the study. The concatenated alignment consists of 1,675 characters (including alignment gaps): 372, 465, and 838 characters used in the *BenA*, *CaM*, and *RPB2*, respectively. The isolates CNUFC JDP37 and CNUFC JDP62 are clustered within the same clade as *T. adpressus* CBS



**Figure 4.** Phylogram generated from the Maximum Likelihood (RAxML) analysis based on a combined *BenA*, *CaM* and *RPB2* sequence data for species classified in the section *Talaromyces*. Bootstrap values  $\geq 70\%$  MLBS and  $\geq 0.90$  BPP are indicated above or below branches. *Talaromyces varians* CBS 386.48 was used as the outgroup. The newly generated sequences are indicated in blue bold. T = ex type.

140620 (ex-type strain) and *T. beijingensis* CBS 140617 (ex-type strain) in the section *Talaromyces*.

### 3.2. Taxonomy

#### 3.2.1. Taxonomy of CNUFC AM-44

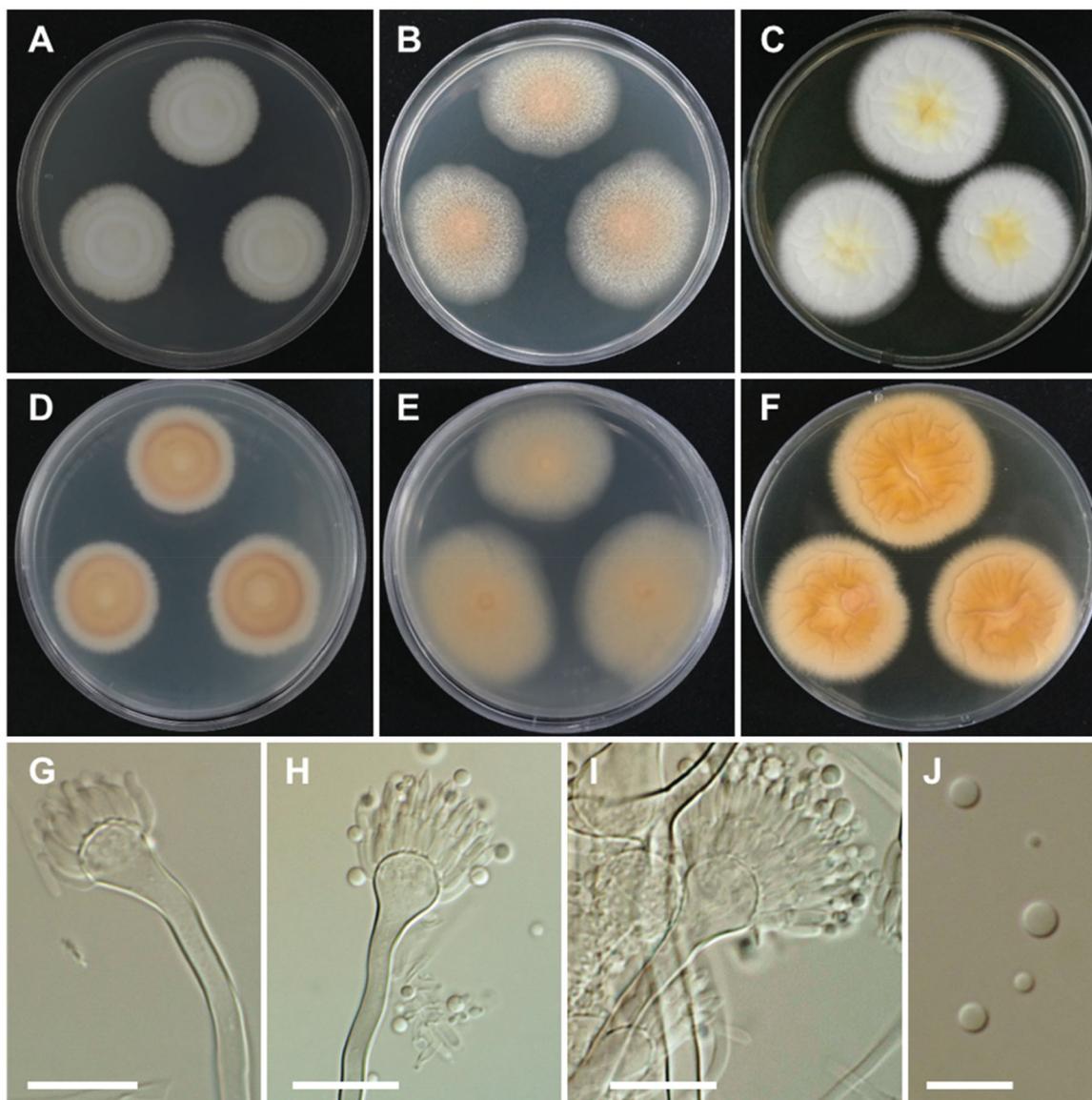
*Aspergillus alabamensis* Balajee, Baddley, Frisvad & Samson, Eukaryotic Cell 8(5): 720 (2009) (Figure 5).

**Colony characteristics:** On CYA, colonies floccose, plane; mycelium white; sporulation moderate; soluble pigments absent; reverse pale yellowish brown; and reached 28–30 mm in diameter after 7 days at 25 °C. On MEA, colonies floccose, plane; mycelium white to yellowish brown at the center; sporulation moderate to dense; soluble pigments absent; reverse pale-yellow brown; and reached 33–35 mm in

diameter after 7 days at 25 °C. On YES, colonies plane, slightly sulcate; mycelium white; sporulation in the colony center; soluble pigments absent; reverse pale orange; and reached 31–33 mm in diameter after 7 days at 25 °C.

**Micromorphology:** Conidiophores biserial, smooth-walled, stipes 3.5–7  $\mu\text{m}$ . Vesicles subglobose, 6–24  $\mu\text{m}$  in diameter. Metulae closely packed, 5.5–10.5  $\times$  2–3  $\mu\text{m}$ . Phialides ampulliform, 5.5–9.5  $\times$  1.5–3  $\mu\text{m}$ . Conidia globose to subglobose, smooth-walled, 2–3  $\mu\text{m}$ .

**Strain examined:** REPUBLIC OF KOREA, Anmyeon-eup (36°34'46.0"N, 126°19'42.7"E), Taean-gun, Chungnam Province, from mud sample, 29 January 2019, H.B. Lee (culture CNUFC AM-44).



**Figure 5.** Morphology of *Aspergillus alabamensis*. A, D: Colonies on Czapek yeast autolysate agar (CYA). B, E: Colonies on malt extract agar (MEA). C, F: Colonies on Yeast extract sucrose agar (YES). (A–C: obverse view, D–F: reverse view). (G–I) Conidiophores; (J) Conidia (scale bars: G–I = 20  $\mu$ m, J = 10  $\mu$ m).

*Note:* CNUFC AM-44 is morphologically similar to *Aspergillus alabamensis* described by Balajee *et al.* [27]. However, CNUFC AM-44 showed to have a partly consistent vesicle size (6–24  $\mu$ m) that is larger than that of *A. alabamensis* CBS 125639 (10–16  $\mu$ m) [27]. Moreover, colony color of CNUFC AM-44 observed on CYA was white, whereas that of CBS 125639 (ex-type strain) was yellowish- to cinnamon-brown [27]. *Aspergillus alabamensis* was isolated from clinical specimens from Alabama, USA [27], and soil samples from Cuba, Argentina, New Mexico, USA, and China [28]. The strain isolated in this study was found in mud.

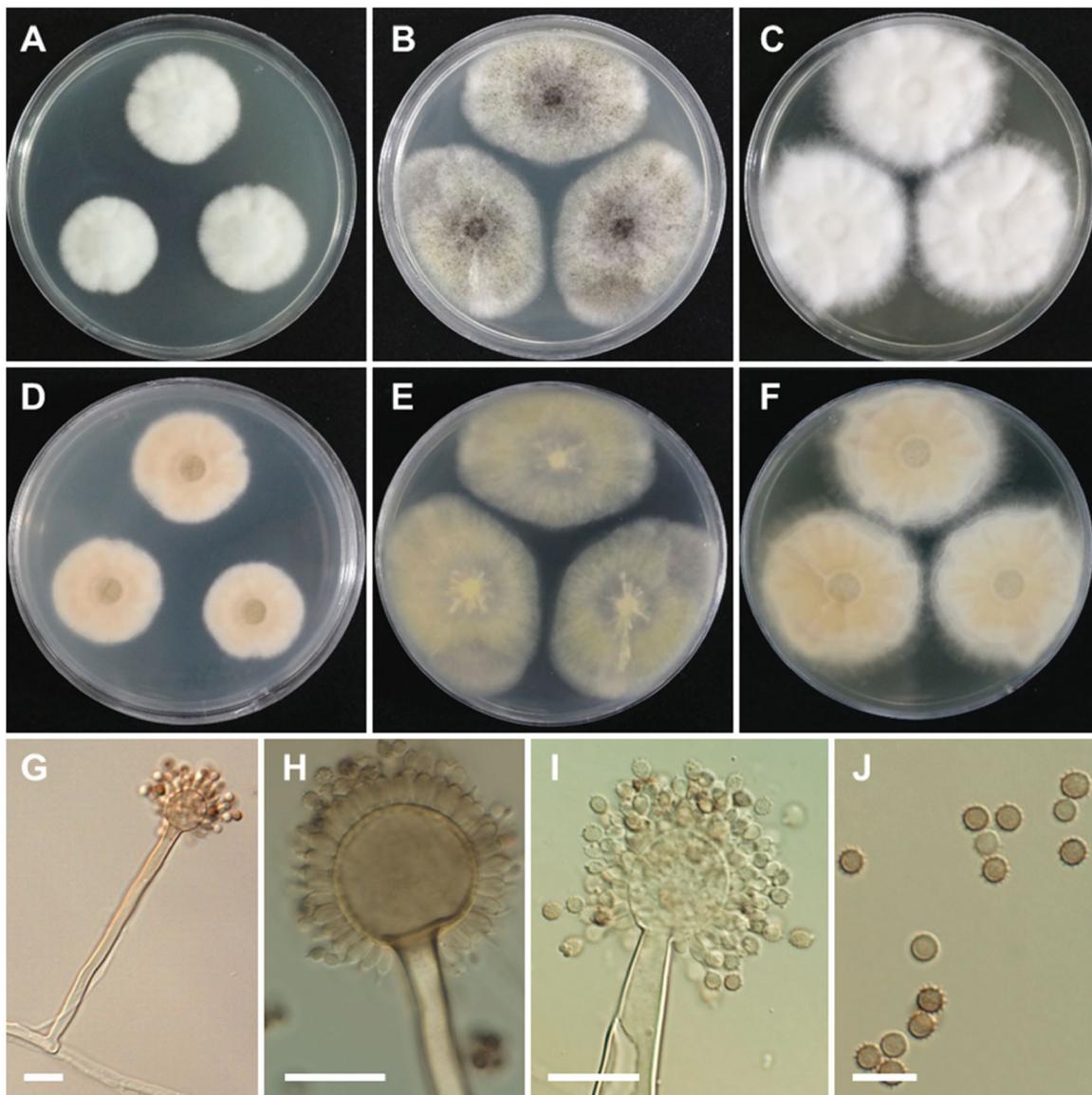
### 3.2.2. Taxonomy of CNUFC JCW3-4

*Aspergillus floridensis* Jurjević, G. Perrone & S.W. Peterson, IMA Fungus 3(2): 169 (2012) (Figure 6).

*Colony characteristics:* On CYA, colonies floccose, moderately radially sulcate; mycelium white;

sporulation poor; soluble pigments absent; reverse pale orange; and reached 24–29 mm in diameter after 7 days at 25 °C. On MEA, colonies floccose, plane; mycelium white to grayish-yellow; conidial heads abundant; sporulation absent; soluble pigments absent; reverse pale yellow; and reached 36–44 mm in diameter after 7 days at 25 °C. On YES, colonies floccose, radially sulcate toward the center; mycelium white; sporulation poor; soluble pigments absent; reverse dull white to pale yellow, and reached 33–41 mm in diameter after 7 days at 25 °C.

*Micromorphology:* Conidiophores uniseriate, smooth-walled, stipes 7.5–15  $\mu$ m. Vesicles globose to subglobose, (8.5–)23.5–50  $\mu$ m in diameter. Phialides cover entire vesicle, 5.5–8.5  $\times$  3–4.5  $\mu$ m. Conidia globose to ellipsoidal, with finely spiny to echinulate surface, and brown at maturity, 3–4.5  $\mu$ m.



**Figure 6.** Morphology of *Aspergillus floridensis*. A, D: Colonies on Czapek yeast autolysate agar (CYA). B, E: Colonies on malt extract agar (MEA). C, F: Colonies on yeast extract sucrose agar (YES). (A–C: obverse view, D–F: reverse view). (G–I) Conidiophores. (J) Conidia (scale bars: G–I = 20  $\mu\text{m}$ , J = 10  $\mu\text{m}$ ).

**Strain examined:** REPUBLIC OF KOREA, Cheonjeyeon (33°15'02.5"N, 126°24'59.6"E) located in Jeju Island, Jeju Province, from waterfall freshwater sample, 18 April 2019, H.B. Lee (culture: CNUFC JCW3-4).

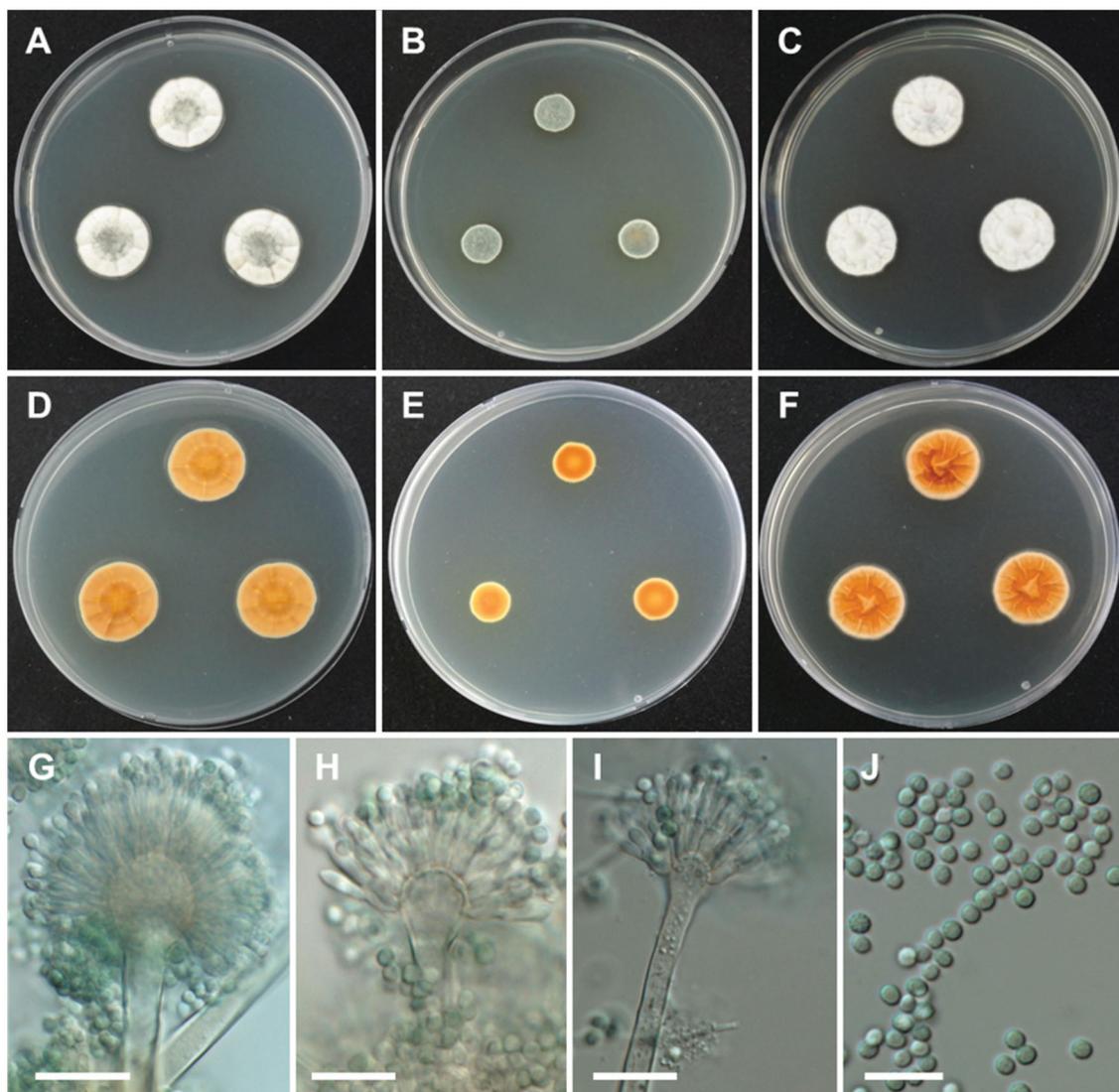
**Note:** CNUFC JCW3-4 also has similar morphological characteristics with the previously described species of *A. floridensis* [29]. However, the vesicles and conidia are slightly smaller than those of *A. floridensis* [29] [Vesicles: (14–)35–65(–105)  $\mu\text{m}$  diam; conidia: 4–5(–6)  $\times$  3.5–5.5  $\mu\text{m}$ ]. In addition, the colony diameter of CNUFC JCW3-4 (24–31 mm) was shorter than that of *A. floridensis* NRRL 62478 (80–85 mm) on CYA [29].

### 3.2.3. Taxonomy of CNUFC S708

*Aspergillus subversicolor* Jurjević, S.W. Peterson & B.W. Horn, IMA Fungus 3(1): 72 (2012). (Figure 7).

**Colony characteristics:** On CYA, colonies plane; mycelium white to gray green at the center, wrinkled, radially sulcate toward the center; sporulation sparse; soluble pigments absent; reverse honey yellow; and reached 20–22 mm in diameter after 7 days at 25 °C. On MEA, colonies plane; mycelium green; sporulation bluish green; soluble pigments absent; reverse brownish orange, and reached 10–12 mm in diameter after 7 days at 25 °C. On YES, colonies plane, radially sulcate; mycelium pale white; sporulation absent; soluble pigments absent; reverse deep orange; and reached 20–23 mm in diameter after 7 days at 25 °C.

**Micromorphology:** Conidiophores biserial, smooth-walled, stipes 4–6.5  $\mu\text{m}$ . Vesicles pyriform or subglobose, 8–17.5(–21.5)  $\mu\text{m}$  in diameter. Metulae, 5–8  $\times$  2.5–4. Phialides ampulliform, 5.5–8(–9)  $\times$  2–3.5  $\mu\text{m}$ . Conidia globose to subglobose, green, 2.5–3.5  $\mu\text{m}$ .



**Figure 7.** Morphology of *Aspergillus subversicolor*. A, D: Colonies on Czapek yeast autolysate agar (CYA). B, E: Colonies on malt extract agar (MEA). C, F: Colonies on yeast extract sucrose agar (YES). (A–C: obverse view, D–F: reverse view). (G–I) Conidiophores; (J) Conidia (scale bars: G–J = 20  $\mu$ m).

*Strain examined:* REPUBLIC OF KOREA, Kunryang-ri (36°44'00.2"N, 126°78'18.0"E), Cheongyang-eup, Cheongyang, Chungnam Province, from the head of bees, 7 May 2020, H.B. Lee (culture: CNUFC S708).

*Note:* The morphological descriptions of CNUFC S708 were matched well with the previously described species of *A. subversicolor* [30]. However, our observations showed that the size of conidia of isolate CNUFC S708 were smaller than previously reported [30]. Colony diameter on CYA media was shown to be different from the previously described *A. subversicolor* (CYA: 22–25 mm after 10 days at 25 °C).

### 3.2.4. Taxonomy of CNUFC JT1301

*Penicillium aquadulcis* Hyang B. Lee & T.T.T. Nguyen, sp. nov.

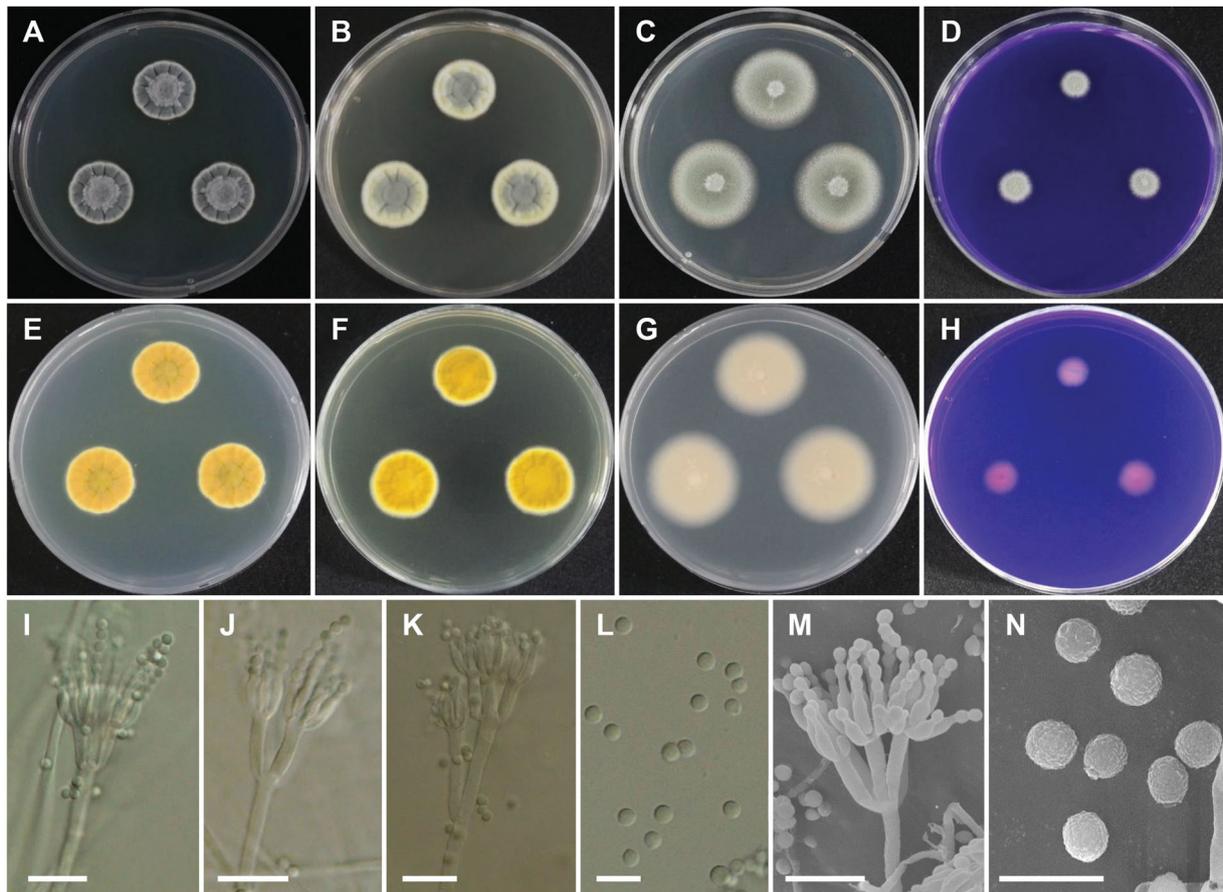
*Index Fungorum:* IF556953; [Figure 8](#).

*Etymology:* *aquadulcis*. Referring to the freshwater source from which the species was first isolated.

*Type specimen:* REPUBLIC OF KOREA, Cheonjeyeon (33°15'02.5"N, 126°24'59.6"E) located in Jeju Island, Jeju Province, from waterfall freshwater sample, 18 April 2019, H.B. Lee (CNUFC HT19009 holotype; ex-type culture: CNUFC JT1301).

Colony diameter after 7 days at 25 °C, in mm: CYA: 16–20 mm; MEA: 27–30; YES: 16–22; CREA: 5–8. CYA, 15 °C: 21–24; CYA, 30 °C: 14–16; CYA, 37 °C: no growth.

*Colony characters:* CYA, 25 °C, 7 days: Colonies elevated in center; radial sulcate, deep; margin entire to slightly irregular; mycelium white; colony texture floccose in center; sporulation moderate; exudate absent; soluble pigment absent; reverse yellow amber. YES, 25 °C, 7 days: Colonies radially sulcate; margins entire; mycelium light yellow; colony texture floccose at center; sporulation moderate to



**Figure 8.** Morphology of *Penicillium aquadulcis* sp. nov. A, E: Colonies on Czapek yeast autolysate agar (CYA). B, F: Colonies on yeast extract sucrose agar (YES). C, G: Colonies on malt extract agar (MEA). D, H: Colonies on creatine sucrose agar (CREA). (A–D: obverse view, E–H: reverse view), (I–L: LM; M, N: SEM). (I–K, M) Conidiophores. (L, N) Conidia (scale bars: I–K, M = 10  $\mu$ m, L, N = 5  $\mu$ m).

good, mainly in center; exudate absent; soluble pigment absent; reverse yellow brown. MEA, 25 °C, 7 days: Colonies plane, slightly raised at center; margins entire; mycelium white; colony texture velvety, slightly floccose in center; sporulation strong; exudate absent; soluble pigment absent; reverse light brown. CREA, 25 °C, 7 days: weak growth, no acid production.

**Micromorphology:** Sclerotia absent. Conidiophores mostly biverticillate, sometimes terverticillate or monoverticillate, with round or smooth walled stipes, non-vesiculate, stipe 2.5–3.5  $\mu$ m wide. Metulae 2–6 per stipe, 8.5–12.5(–16)  $\times$  2.5–3.5  $\mu$ m. Phialides flask-shaped, 3–10 per metula, 7–10.5  $\times$  2–3  $\mu$ m. Conidia in short chains, finely rough walled, globose to subglobose, 2–2.5  $\mu$ m.

**Note:** Our phylogenetic analyses showed that *P. aquadulcis* forms a separate lineage, phylogenetically related to *P. manginii*. However, slow growth when cultivated on CYA, MEA, CREA is observed and the property can be used to easily distinguish the new species from these species. The conidial shape of *P. aquadulcis* (globose to subglobose) is different from that of *P. manginii* (ellipsoidal). Additionally,

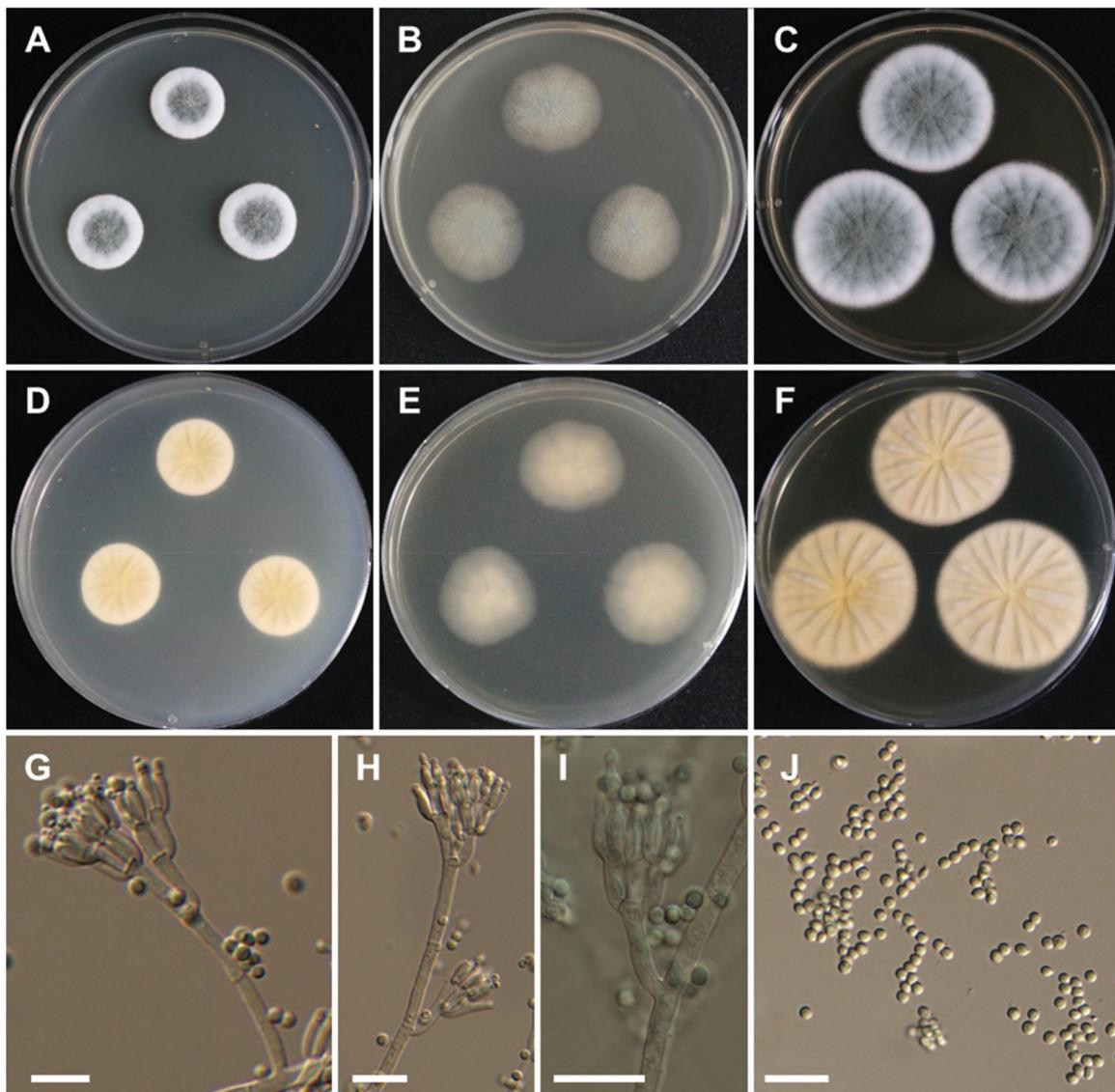
*P. manginii* is known to produce sclerotia, while *P. aquadulcis* does not.

**Additional material examined:** REPUBLIC OF KOREA, Cheonjeyeon (33°15'02.5"N, 126°24'59.6"E) located in Jeju Island, Jeju Province, from waterfall freshwater sample, 18 April 2019, H.B. Lee (culture: CNUFC JT1302).

### 3.2.5. Taxonomy of CNUFC WT202

*Penicillium flavigenum* Frisvad & Samson, Mycological Research 101(5): 622 (1997) (Figure 9).

**Colony characteristics:** On CYA, colonies floccose, radially sulcate; mycelium white; exudate absent; soluble pigments absent; sporulation moderate; reverse pale yellow; and reached 19–21 mm in diameter after 7 days at 25 °C. On MEA, colonies plane; exudate absent; soluble pigments absent; sporulation strong; reverse light yellow; and reached 24–27 mm in diameter after 7 days at 25 °C. On YES, colonies plane, radially sulcate; mycelium white; exudate absent; soluble pigments absent; sporulation moderate; reverse pale yellow; and reached 35–38 mm in diameter after 7 days at 25 °C.



**Figure 9.** Morphology of *Penicillium flavigenum*. A, D: Colonies on Czapek yeast autolysate agar (CYA). B, E: Colonies on malt extract agar (MEA). C, F: Colonies on yeast extract sucrose agar (YES). (A–C: obverse view, D–F: reverse view). (G–I) Conidiophores; (J) Conidia (scale bars: G–J = 20  $\mu$ m).

**Micromorphology:** Conidiophores terverticillate, stipes, 2–5  $\mu$ m. Metulae were cylindrical, 7–11  $\times$  2.5–3.5  $\mu$ m. Phialides were cylindrical, 5.5–8.5  $\times$  2–3  $\mu$ m. Conidia smooth-walled, globose to subglobose, 2.5–3.5  $\mu$ m.

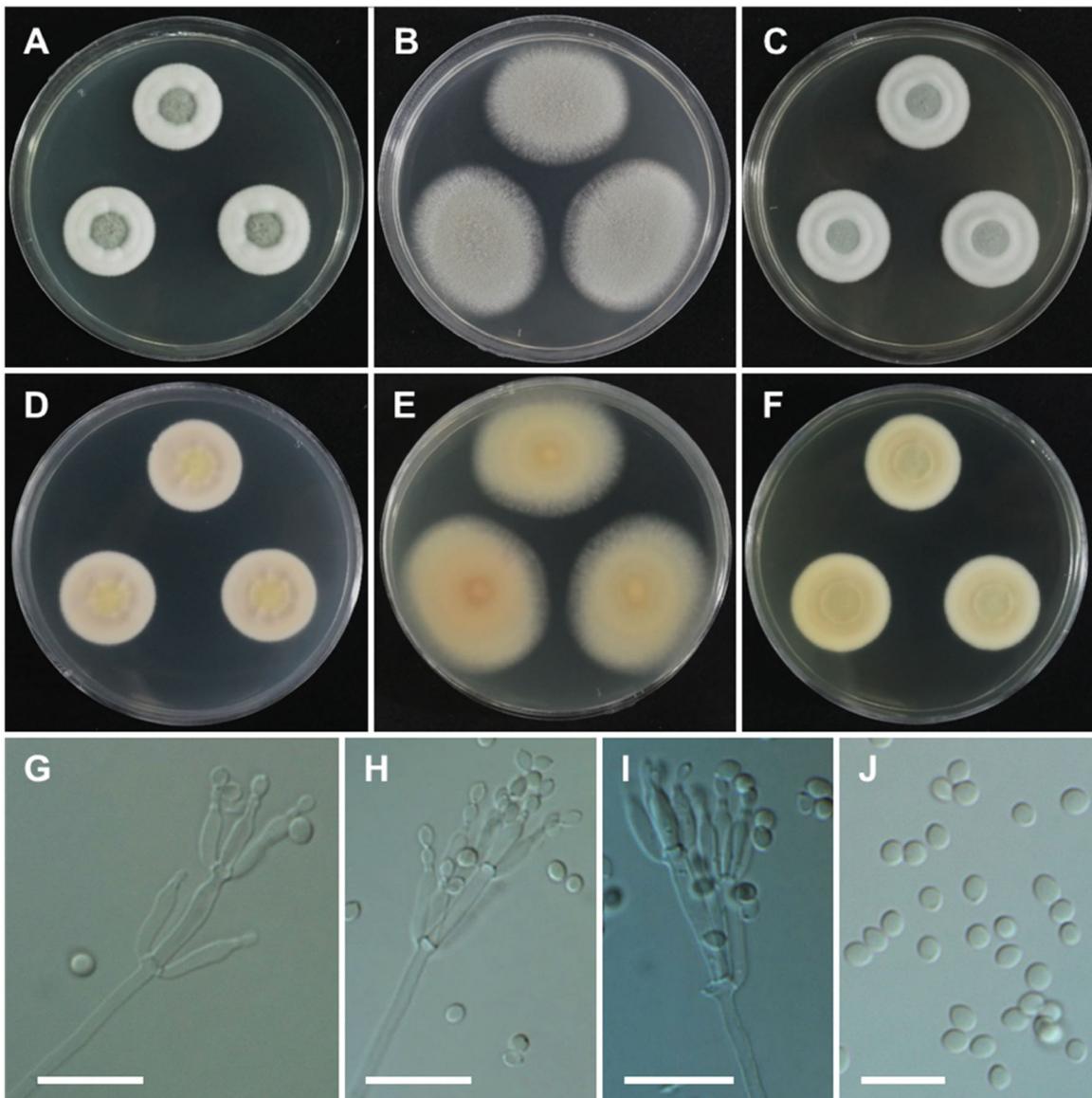
**Strain examined:** REPUBLIC OF KOREA, Kunryang-ri (36°44'00.2"N, 126°78'18.0"E), Cheongyang-eup, Cheongyang, Chungnam Province, from freshwater sample, 1 June 2020, H.B. Lee (culture: CNUFC WT202).

**Note:** There were differences observed in the colony diameter for CNUFC WT202 in comparison to previous descriptions. Its colony diameter was different from that of previously described *Penicillium flavigenum* on CYA (21–31 mm) [31]. In this isolate, conidia were slightly smaller than *Penicillium flavigenum* (2.5–4  $\mu$ m) [31].

### 3.2.6. Taxonomy of CNUFC AS1-29

*Penicillium laevigatum* L. Cai, Houbraken, R.N. Barbosa & X.Z. Jiang, Cladistics 35(5): 537 (2018) (Figure 10).

**Colony characteristics:** On CYA, colonies slightly sulcate; margins entire; mycelium white; sporulation sparse; soluble pigments absent; exudates absent; reverse light grayish yellow; and reached 21–23 mm in diameter after 7 days at 25 °C. On MEA, mycelium white; slightly raised at the center; sporulation strong; soluble pigments absent; exudates absent; reverse light orange yellow; and reached 36–40 mm in diameter after 7 days at 25 °C. On YES, colonies slightly raised at the center; margins entire; mycelium white; sporulation sparse; soluble pigments absent; exudates absent; reverse buff-yellow; and reached 23–25 mm in diameter after 7 days at 25 °C.



**Figure 10.** Morphology of *Penicillium laevigatum*. A, D: Colonies on Czapek yeast autolysate agar (CYA). B, E: Colonies on malt extract agar (MEA). C, F: Colonies on yeast extract sucrose agar (YES). (A–C: obverse view, D–F: reverse view). (G–I) Conidiophores; (J) Conidia (scale bars: G–I = 20  $\mu$ m, J = 10  $\mu$ m).

**Micromorphology:** Conidiophores mono and biverticillate, stipes, 2–4  $\mu$ m. Metulae 2–3 per stipe, appressed to divergent, 8.5–19  $\times$  2–4  $\mu$ m. Phialides ampulliform, 6–17  $\times$  2–3.5  $\mu$ m. Conidia smooth-walled, globose to subglobose, 2–3  $\mu$ m.

**Strain examined:** REPUBLIC OF KOREA, Anmyeon-eup (36°34'46.0"N, 126°19'42.7"E), Taean-gun, Chungnam Province, from soil sample, 29 January 2019, H.B. Lee (culture CNUFC AS1-29).

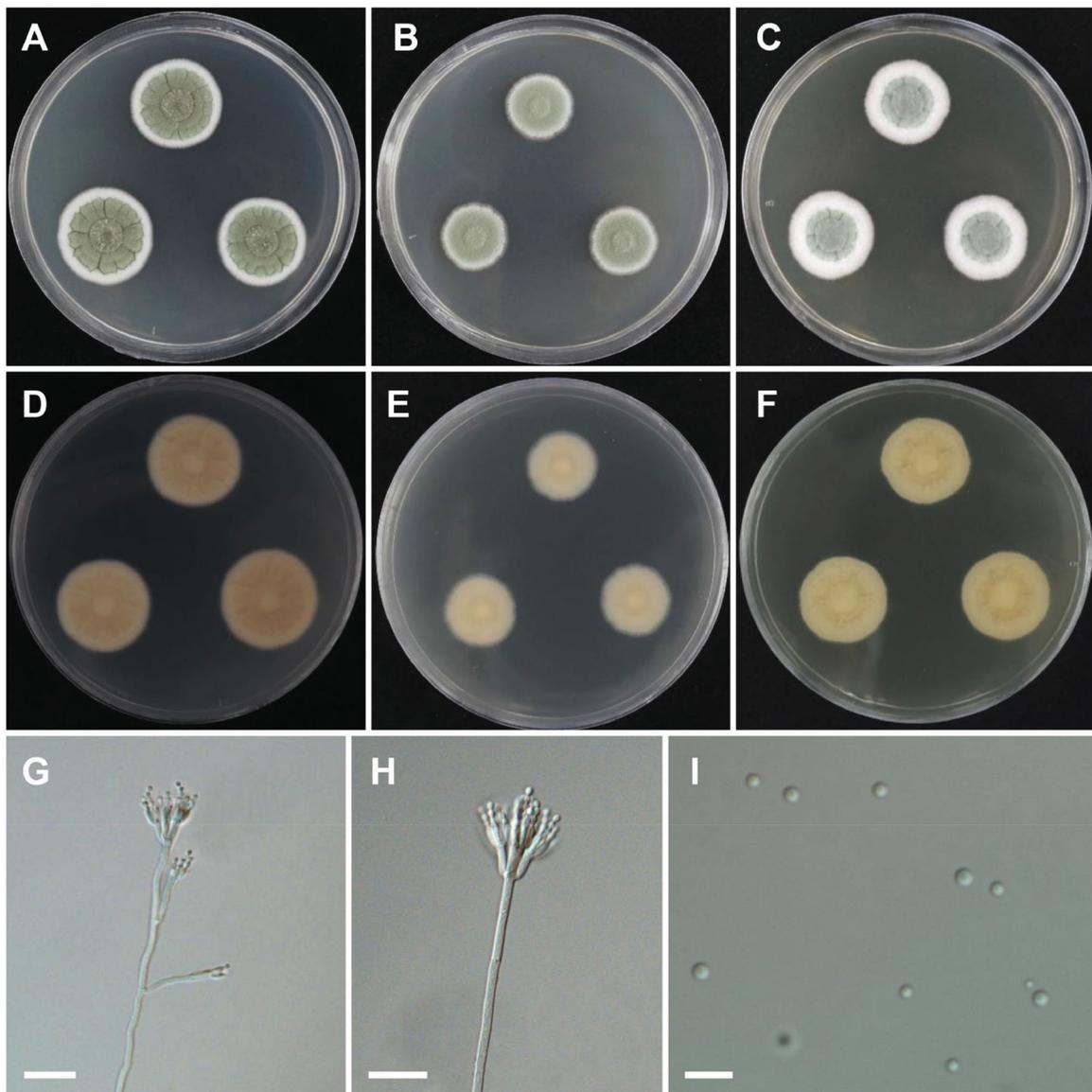
**Note:** Based on phylogenetic analysis of combined *BenA*, *CaM*, and *RPB2* sequence data, our isolate CNUFC AS1-29 was clustered with the ex-type strains of *P. laevigatum* and *P. wandoense* in *laevigatum* clade (Figure 3). The colony morphology and culture characteristics of CNUFC AS1-29 strain were similar to the previous descriptions [32]. As reported by Houbraeken *et al.* [11], *P. laevigatum* and *P. wandoense*, which were isolated from freshwater collected at Wando, Korea [33], were

conspicuous. However, *P. wandoense* (3–5  $\mu$ m) differs from *P. laevigatum* (1–14  $\mu$ m) by having smaller number of phialides per metulae [32]. *Penicillium wandoense* also differs from *P. laevigatum* (type species) and isolate CNUFC AS1-29 in terms of reverse color on MEA. Thus, future morphology and molecular studies are needed to confirm the relationship between *P. laevigatum* and *P. wandoense*.

### 3.2.7. Taxonomy of CNUFC JCW3-5

*Penicillium lenticrescens* Visagie, Seifert & Samson, *Studies in Mycology* 78: 123 (2014) (Figure 11).

**Colony characteristics:** On CYA, colonies sulcate; margins entire; mycelia white; sporulation moderate; soluble pigments absent; exudates absent; reverse pale yellow; and reached 23–29 mm in diameter after 7 days at 25 °C. On MEA, colonies slightly raised at the center, plane; mycelium white; soluble pigments absent; exudates absent; reverse yellowish gray; and reached



**Figure 11.** Morphology of *Penicillium lenticrescens*. A, D: Colonies on Czapek yeast autolysate agar (CYA). B, E: Colonies on malt extract agar (MEA). C, F: Colonies on yeast extract sucrose agar (YES). (A–C: obverse view, D–F: reverse view). (G, H) Conidiophores; (I) Conidia (scale bars: G, H = 20, I = 10  $\mu$ m).

16–18 mm in diameter after 7 days at 25 °C. On YES, colonies sulcate; margins entire; mycelia white; slightly raised at the center; sporulation absent; soluble pigments absent; exudates absent; reverse greenish yellow; and reached 19–23 mm in diameter after 7 days at 25 °C.

**Micromorphology:** Conidiophores biverticillate and divaricate, stipes smooth, 2.5–3.5  $\mu$ m. Metulae divergent, 8.5–14.5  $\times$  2–4  $\mu$ m. Phialides ampulliform, 7.5–12  $\times$  2–3.5  $\mu$ m. Conidia globose to subglobose, 2–3  $\mu$ m.

**Strain examined:** REPUBLIC OF KOREA, Cheonjeyeon (33°15'02.5"N, 126°24'59.6"E) located in Jeju Island, Jeju Province, from waterfall freshwater sample, 18 April 2019, H.B. Lee (culture: CNUFC JCW3-5).

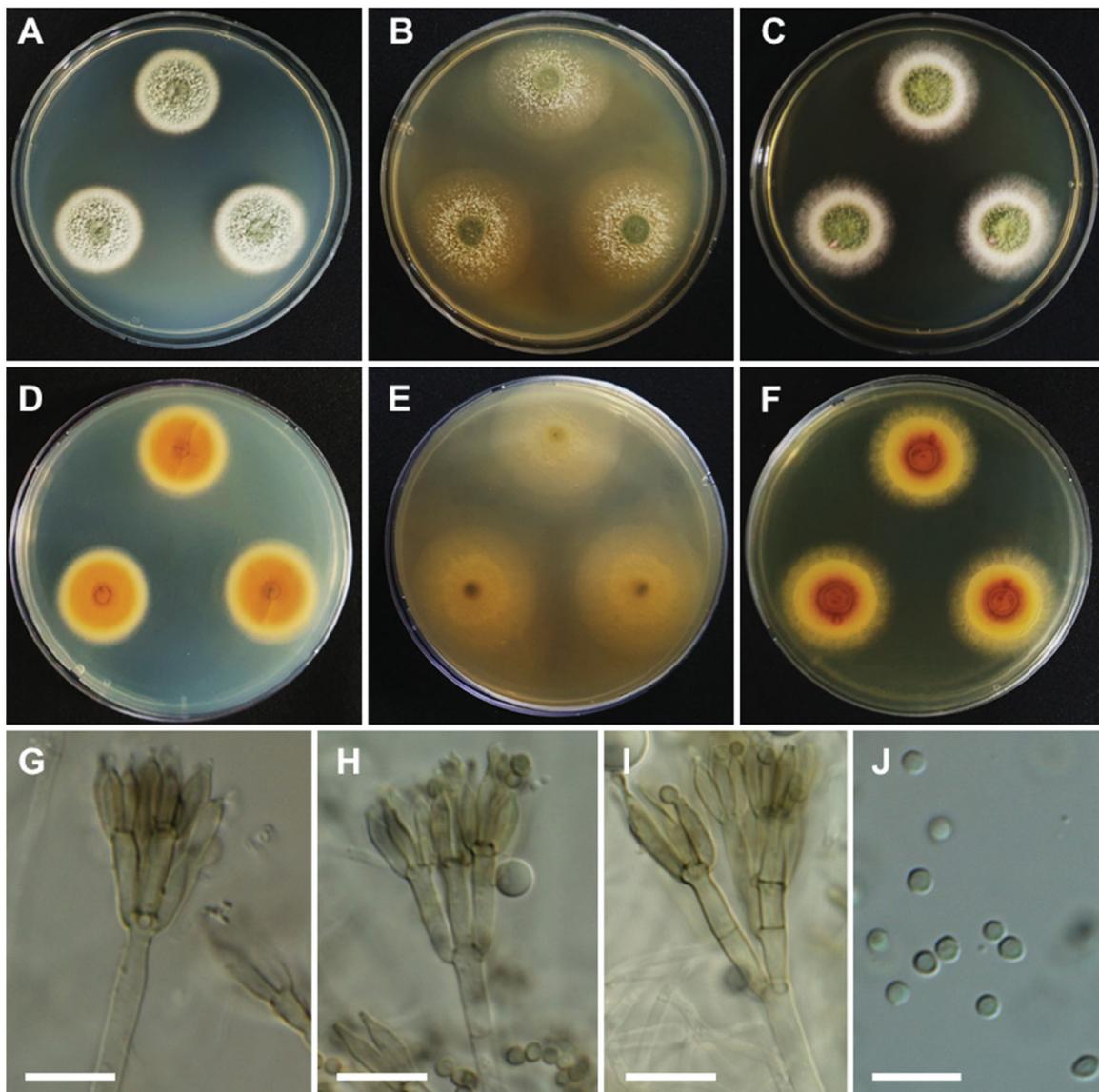
**Note:** The morphological characteristics of the isolate were consistent with those of *P. lenticrescens*, as described by Visagie *et al.* [2]. However, our strain produced biverticillate and divaricate

conidiophores, in contrast to the mostly biverticillate sporangiophores of *P. lenticrescens* described by Visagie *et al.* [2].

### 3.2.8. Taxonomy of CNUFC JDP37

*Talaromyces adpressus* A.J. Chen, Frisvad & Samson, Studies in Mycology 84: 124 (2016) (Figure 12).

**Colony characteristics:** On CYA, colonies slightly sulcate; margins entire; mycelium white; texture floccose; sporulation moderately to dense; soluble pigments absent; exudates absent; reverse yellowish orange, and reached 22–24 mm in diameter after 7 days at 25 °C. On MEA, colonies slightly raised at the center, plane; mycelium white; sporulation sparse; texture floccose in the center; soluble pigments absent; exudates absent; reverse light yellow; and reached 32–34 mm in diameter after 7 days at 25 °C. On YES, colonies raised at the center; appearance green; margins entire; mycelium white;



**Figure 12.** Morphology of *Talaromyces adpressus*. A, D: Colonies on Czapek yeast autolysate agar (CYA). B, E: Colonies on malt extract agar (MEA). C, F: Colonies on yeast extract sucrose agar (YES). (A–C: obverse view, D–F: reverse view). (G–I) Conidiophores; (J) Conidia (scale bars: G–J = 10 µm).

sporulation moderate; soluble pigments absent; exudates absent; reverse brownish orange and light yellow; and reached 28–30 mm in diameter after 7 days at 25 °C.

**Micromorphology:** Conidiophores biverticillate, stipes smooth, 2.5–4 µm. Metulae 2–5, 9–16 × 2.5–3.5 µm. Phialides 2–5, 8.5–12 × 2–3 µm. Conidia smooth, globose to subglobose or ellipsoidal, 2–4 µm.

**Strain examined:** REPUBLIC OF KOREA, Mokpo (34°49'17.5"N, 126°24'35.0"E), Jeonnam province, from freshwater sample, September 2020, H.B. Lee (culture: CNUFC JDP37).

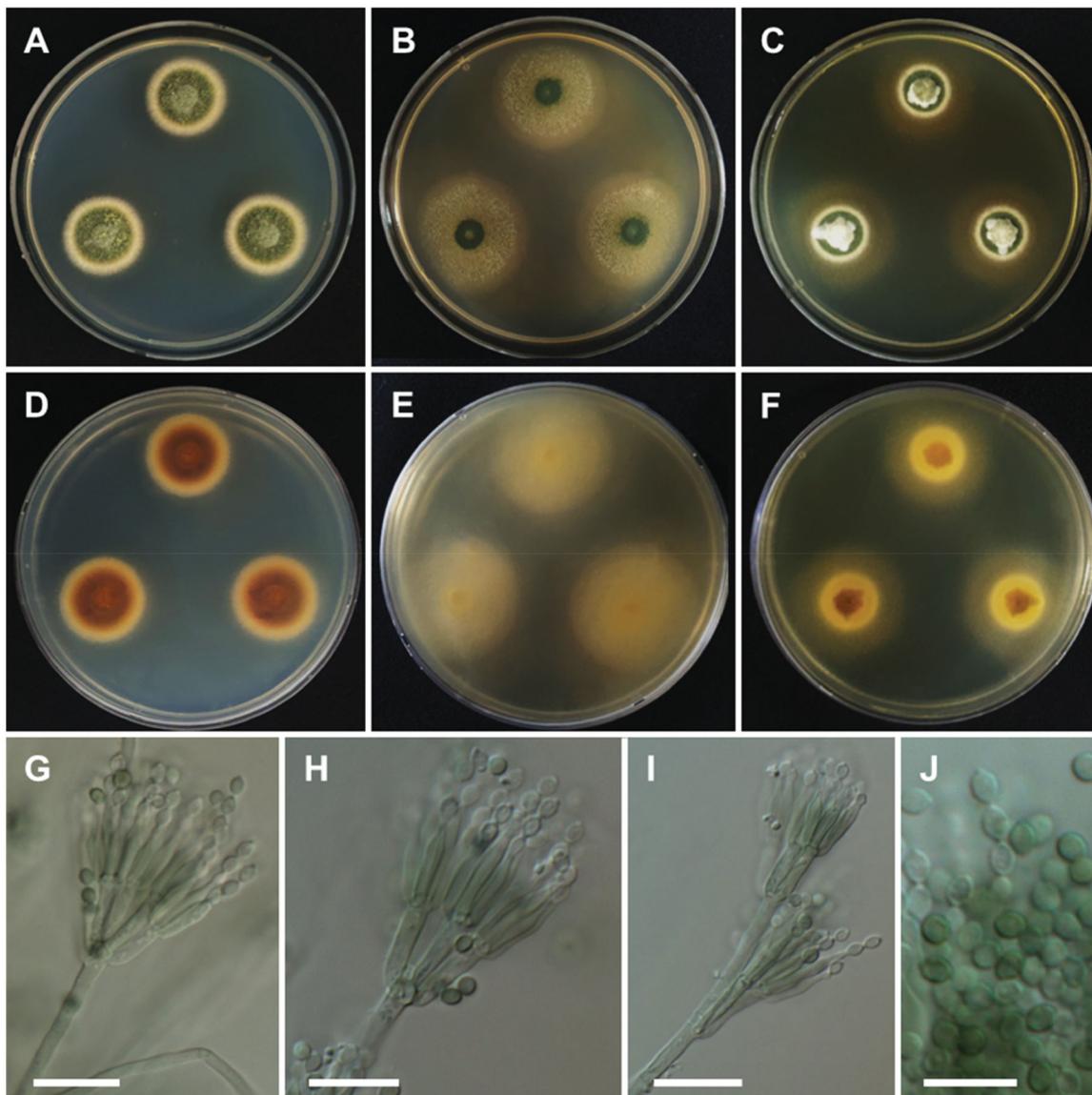
**Note:** The morphological characteristics of the isolate CNUFC JDP37 in this study were similar to those of the previously described *T. adpressus* by Chen *et al.* [34]. However, our observations showed that the size of conidia of the isolate CNUFC JDP37 was smaller than that of previously reported species.

### 3.2.9. Taxonomy of CNUFC JDP62

*Talaromyces beijingensis* A.J. Chen, Frisvad & Samson, *Studies in Mycology* 84: 125 (2016) (Figure 13).

**Colony characteristics:** On CYA, colonies moderately deep, raised at the center; margins entire; mycelium white; texture velvety; sporulation dense; soluble pigments absent; reverse peach; and reached 26–29 mm in diameter after 7 days at 25 °C. On MEA, colonies slightly raised at the center, plane; mycelium white; sporulation sparse; reverse light yellow; and reached 31–33 mm in diameter after 7 days at 25 °C. On YES, colonies raised at the center; appearance green; margins entire; mycelium white; sporulation moderate; soluble pigments absent; exudates absent; reverse brownish orange at the center, yellow at the edge; and reached 29–31 mm in diameter after 7 days at 25 °C.

**Micromorphology:** Conidiophores mostly terverticillate, sometimes biverticillate, stipes smooth, 2.5–3.5 µm.



**Figure 13.** Morphology of *Talaromyces beijingensis*. A, D: Colonies on Czapek yeast autolysate agar (CYA). B, E: Colonies on malt extract agar (MEA). C, F: Colonies on yeast extract sucrose agar (YES). (A–C: obverse view, D–F: reverse view). (G–I) Conidiophores; (J) Conidia (scale bars: G–J = 10  $\mu$ m).

Metulae 2–5, 10–14  $\times$  2.5–3.5  $\mu$ m. Phialides 9.5–13  $\times$  2.0–3.0  $\mu$ m. Conidia smooth, ellipsoidal to fusiform, 2.5–3.5  $\times$  2–2.5  $\mu$ m. Ascospores not observed.

*Strain examined:* REPUBLIC OF KOREA, Mokpo (34°49'17.5"N, 126°24'35.0"E), Jeonnam province, from freshwater sample, September 2020, H.B. Lee (culture: CNUFC JDP62).

*Note:* The morphological characteristics of the isolate CNUFC JDP62 in this study were similar to those of the previously described *T. beijingensis* by Chen *et al.* [34]. However, the number of metulae are larger than those of *T. beijingensis* and smaller conidia [34]. In addition, differences in colony diameter were observed on MEA (35–39 mm) [34].

#### 4. Discussion

Recent studies on the genera *Aspergillus*, *Penicillium*, and *Talaromyces* have been conducted

in Korea, yet their distribution is not fully known, with various areas remain unexplored. In this study, a new species and eight new records are described and illustrated. The ITS is the DNA barcoding marker for the identification of fungi. However, many of the previous studies have shown that the only ITS is insufficient for *Aspergillus*, *Penicillium*, and *Talaromyces* identification as closely related species often share similar or identical sequences. The *BenA*, and *CaM* sequences have been used for the identification of these three genera, as this is the recommended identification marker [2,3,11]. In this study, the multigene phylogeny proposed by Houbraken *et al.* [11] allowed us to recognize a new species, and to identify eight recently described in the genera *Aspergillus*, *Penicillium* and *Talaromyces*. A new species described here belongs to section *Citrina*, series *Westlingiorum*. *Penicillium aquadulcis* is closely related to *P. manginii*, and are in one

clade with *Penicillium* sp. SFC P0237 and *Penicillium* sp. SFC P0230. *Penicillium* sp. SFC P0237 and *Penicillium* sp. SFC P0230 were isolated from *Rhododendron brachycarpum* in Korea but there are no descriptions for these species [35]. *Penicillium aquadulcis* differs from *P. manginii* by having smaller colonies on CYA, MEA, and CREA. Conidial shape and production of sclerotia can be easily used to distinguish between *Penicillium aquadulcis* and *P. manginii*. Section *Citrina* currently includes 41 species and 9 series (ser. *Gallaica*, ser. *Roseopurpurea*, ser. *Euglauca*, ser. *Citrina*, ser. *Paxillorum*, ser. *Sheariorum*, ser. *Sumatraensia*, ser. *Copticularum* and ser. *Westlingiorum*) [11]. Twenty-two species are currently accepted in series *Westlingiorum* and most of those species isolated from soil [36]. In this study, we isolated a novel species from freshwater.

Hawksworth [37] estimated that there are approximately 1.5 million fungal species on Earth. Around 3,000 species are known to be associated with aquatic habitats, with more than 600 species of ascomycetes reported from freshwater habitats [38,39]. The importance of these organisms in secondary metabolite production has also been recognized. However, the knowledge on freshwater fungi remains insufficient. Moreover, these species are less explored than terrestrial fungi.

In this study, both *T. adpressus*, and *T. beijingensis* were also found in freshwater. *Talaromyces adpressus* and *T. beijingensis* are known *Talaromyces* species, which were originally described from air in China. To the best of our knowledge, species from aquatic environments have been reported for the sections, *Helici*, *Purpurei* and *Talaromyces* [40,41]. Further studies are necessary to understand both the distribution and relevance of species in the section *Talaromyces* in aquatic environments.

Insects are known to host various fungi [16,42,43]. Recently, Barbosa *et al.* [42] reported an association of *Penicillium* and *Talaromyces* with stingless bees. Notably, we also isolated *A. subversicolor* from bee niche. Previous studies isolated *A. subversicolor* from coffee berries and as endophytes from macroalgae [30,44], suggesting that this species has a broad ecological niche. The occurrence and distribution of *Aspergillus* associated with insects in freshwater habitats remain unknown. Further studies on the relationship of fungi and insects are thus needed to understand the benefits that fungi may provide to their hosts. In addition, the genera *Aspergillus*, *Penicillium*, and *Talaromyces* are now known to produce secondary metabolites, warranting further studies on the biodiversity of these fungi and their potential applications in biotechnology.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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